

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
14 March 2002 (14.03.2002)

PCT

(10) International Publication Number
WO 02/20555 A2

- (51) International Patent Classification⁷: C07K (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (21) International Application Number: PCT/NO01/00363
- (22) International Filing Date:
3 September 2001 (03.09.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
2000 4412 4 September 2000 (04.09.2000) NO
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- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NI, SN, TD, TG).
- Published:
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 02/20555 A2

(54) Title: HIV REGULATORY AND AUXILIARY PEPTIDES, ANTIGENS, VACCINE COMPOSITIONS, IMMUNOASSAY KIT AND A METHOD OF DETECTING ANTIBODIES INDUCED BY HIV

(57) Abstract: The present invention comprises novel and modified peptides capable of inducing a HIV-1 specific immune response without antagonizing the cytotoxic T-cell activity in order to achieve an effective prophylactic and therapeutic vaccine against HIV. The peptides are based on conserved regions of HIV Tat and Rev, regulatory proteins and Nef, auxiliary proteins. Antigens in free- or carrier-bound form comprising at least one of the said peptides, vaccine compositions containing at least one of the antigens, immunoassay kits and a method of detecting antibodies induced by HIV or HIV specific peptides using such antigens, are described.

Title : HIV regulatory and auxiliary peptides, antigens, vaccine compositions, immunoassay kit and a method of detecting antibodies induced by HIV."

The present invention relates to novel peptides based on conserved regions of the regulatory and auxiliary HIV proteins, antigens in free or carrier-bound form comprising at least one of the said peptides, vaccine compositions containing at least one of the antigens, immunoassay kits and a method of detecting antibodies, induced by human immunodeficiency virus (HIV) or HIV-specific peptides, using such antigens.

BACKGROUND

Human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS) continues to present a formidable challenge to health in developing countries. In the Western world, therapeutic strategies that target HIV-1 replication and maturation have had a prominent impact on disease progression. The high cost of current treatment, high toxicity of the drugs and lack of cure, however, means that the development of safe and effective vaccines remains paramount for control of the AIDS pandemic.

HIV-1 is a complex retrovirus encoding six regulatory and auxiliary genes not found in the simple retroviruses, namely, *tat*, *rev*, *nef*, *vif*, *vpr* and *vpu* (Table 1). In eukaryotic cells, only completely spliced mRNAs are exported to the cytoplasm for translation. Unspliced or partially spliced RNAs are retained and eventually degraded in the nucleus. In this way, proteins encoded by the *tat*, *rev* and *nef* genes (designated Tat, Rev and Nef) derived from multiply spliced RNA species, are expressed first and constitute early HIV-1 gene expression. In order to express singly spliced RNAs and also to transport the full length unspliced RNA genome into the cytoplasm for packaging, HIV-1 has developed means to overcome the restrictions on RNA transport.

The regulatory proteins, Tat and Rev, are essential for HIV-1 replication since mutations in these proteins eliminate HIV-1 production (Dayton A.I., et.al. (1986) Cell, 44:941-947, and Fisher, A.G., et.al. (1986) Nature, 320:367-371.).

The auxiliary genes are derived from exons positioned entirely upstream of the HIV-1 envelope gene e.g. *vif* or exons upstream of as well as within *env* but in different reading frames e.g. *tat*, *rev*. The efficiency of splicing in part regulates the levels of gene expression of the different auxiliary proteins.

Following integration of HIV-1 proviral DNA, predominantly truncated forms of mRNA are synthesised by the cellular RNA polymerase II which interacts with sites on the 5'

long terminal repeat (LTR) of the proviral DNA. *tat* is one of the first genes to be expressed and carries a nuclear localisation signal. It is a potent transcriptional activator that enhances LTR-directed transcription up to a thousand fold. Continuous expression of Tat ensures a positive feed back loop for continued high level gene expression. Unlike conventional transcriptional activators that interact with DNA sequences, Tat binds directly to the 5' ends of all HIV-1 RNAs at a specific stem-loop secondary structure, TAR (transactivating response element). The structure of the loop is highly conserved and essential for Tat function. The structure of the Tat/TAR interaction has been analysed using nuclear magnetic resonance (NMR) (Puglisi et al. (1992) Science, 257:76-80). Tat binds TAR in association with the cellular protein, cyclin T, which in turn binds CDK9 that phosphorylates the RNA polymerase II C-terminal domain, thereby promoting the elongation of RNA transcripts. These Tat cellular cofactors are only present in activated cells, their absence represses transcription of proviral DNA resulting in a 'quasi latency' in T lymphocytes.

Tat is expressed from two exons, both the nuclear localisation signal and the TAR binding region are located in the first exon. Tat is secreted from infected cells and can exert heterologous effects on neighbouring cells. These include cellular activation (Hofman et al. (1993) Blood, 82:2774-2780.), induction of cellular apoptosis (Macho et al. (1999) Oncogene, 18:7543-7551.1999) functioning as a secretable growth factor (Trinh, D.P. et.al. (1999) Biochem. Biophys. Res. Commun., 256:299-306.) and modulating host cell protein synthesis in favour of viral protein synthesis (Xiao et al. (1998) Biochem. Biophys. Res. Commun., 244:384-389.1998). Therapeutic strategies targeting Tat will therefore have a strong impact on HIV-1 infection.

The small multiply spliced mRNAs encoding Tat, Rev and Nef predominate during early phase after infection. When a threshold level of Rev is produced, unspliced and singly spliced RNAs accumulate in the cytoplasm for translation, allowing productive infection to proceed. Failure to generate this threshold level of Rev may contribute to HIV quasi latency. Rev can only bind to RNAs carrying an RNA structure, RRE (Rev responsive element) which is located in the *env* coding region of the genome. Rev interacts with the RRE as a multimer through a basic arginine rich region present in the amino terminal half of the protein. A consequence of this interaction is the transport of partially spliced RNAs that will provide gene products such as Env, Vif, Vpu and Vpr as well as unspliced RNAs that will serve as new genomes for incorporation into assembling particles. Structural analysis of the Rev/RRE interaction has also been carried out using NMR (Battiste et al. (1996) Science, 273:1547-1551.1996). Rev carries a leucine

rich export signal that allows it to shuttle between the nucleus and the cytoplasm for continued transport of newly synthesised RNAs (Kalland et al. (1994). J. Virol., 68:1475-1485; Meyer & Malim, (1994) Genes Dev., 8:1538-1547.). In this way, Rev ensures that the structural genes are expressed late following the regulatory genes.

5 Therapeutic strategies directed against Rev will interrupt the viral life cycle early in infection.

Although originally described as a negative factor, Nef has later been shown to have positive effects on virus replication and is expressed in larger quantities than that of Tat -

10 and Rev, both early and throughout infection. Nef is myristylated at the N-terminus and is associated with the inner side of the plasma membrane. Nef is partly responsible for down regulation and degradation of surface CD4 by endocytosis (Piguet et al. (1998) EMBO J., 17:2472-2481.). Removing CD4 from the cell surface prevents superinfection with other HIV-1 strains, or reinfection with newly released virus. Nef is also responsible

15 for the down regulation of MHC class I thereby protecting infected cells from destruction by cytotoxic T-lymphocytes (Le Gall et al. (1997). Res. Virol., 148:43-47.). Nef is not an essential viral protein since it is not required for *in vitro* infection of peripheral blood lymphocytes or T-cell lines. Nef deletion mutants, however, are less pathogenic over long periods of time. Nef also has complex effects on signal transduction pathways in

20 the cell and contains a proline rich region that can interact with the SH3 domain of kinases involved in T-cell activation, a feature necessary for efficient HIV replication (Moarefi et al. (1997). Nature, 385:650-653.). Nef containing viruses are capable of more viral DNA synthesis than viruses deleted in the Nef gene which suggests that Nef directly or indirectly activates the viral reverse transcriptase. The low level of Nef

25 associated with virions may be responsible for this phenomenon. Low levels of Nef are also released from infected cells although the potential effect on neighbouring cells is unclear. Since Nef is expressed early in infection and has significant effects on CD4 and MHC class I expression as well as disease progression, it represents an important target for future therapeutic strategies.

30 Tat and Nef are secreted and can be taken up by macrophages and expressed in association with MHC class II molecules. This improves their suitability as targets for peptide based therapies which also would be expressed in the context of MHC class II. It is clear that targeting early gene products that are essential for HIV-1 replication, such as Tat and Rev should be given priority in addition to Nef which is also expressed early

35 and influences disease progression.

Table 1 HIV-1 Regulatory and auxiliary proteins.

Gene	Protein	Name	Expression	Localisation	Functions
<i>Tat</i>	Tat	Transactivator of viral transcription	Early	Nucleus	Activates viral transcription. Secreted from infected cells where it can activate T-cells, induce apoptosis and function as a growth factor.
<i>Rev</i>	Rev	Nuclear RNA export factor	Early	Nucleolus, nucleoplasm, cytoplasm	Regulates splicing/RNA transport to the cytoplasm. A shuttle protein.
<i>Nef</i>	Nef	Numerous effector functions	Early	Cytoplasm, membrane associated. Virions	Triggers CD4 endocytosis, Down regulates MHC class 1 expression. Binds to kinases and may influence T-cell signalling and activation.
<i>Vpu</i>	Vpu	Viral protein u	Late	Cytoplasm, membrane associated	Triggers intracellular CD4 degradation, down regulates MHC class 1, nonspecific promoter of retroviral particle release.
<i>Vif</i>	Vif	Viral infectivity factor	Late	Cytoplasm, membranes Virions	Enhances infectivity of viral particles in a cell dependent manner. Improves viral DNA synthesis during reverse transcription.
<i>Vpr</i>	Vpr	Viral protein r	Late	Predominantly nucleus Virions	Contributes to nuclear import of preintegration complex. Arrests cells in G2/M phase of the cell cycle.

5

Naturally occurring HIV sequences in vaccine candidates are not capable of stimulating a stable longterm immune response due to HIVs inherent ability to hide by changing the appearance of the epitopes presented for the immune system. To overcome this variable presentation of epitopes, certain amino acid substitutions and amino acid combinations will support the immune system to present and recognize these foreign virus antigens in a reliable manner and thus to a greater extent.

10

Based on the above background, we decided to investigate the possibility

of designing novel synthetic peptides which can mimic the epitopes from the regulatory and auxiliary HIV proteins in such a way that they can be exposed for both the humoral as well as the cellular part of the immune system, to meet the need for an effective therapeutic and/or prophylactic vaccine.

5

The initial work was based on the native Tat amino acid sequences published by Korber B., et al., Human Retroviruses and AIDS 1997 Eds. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM. The first Tat epitope is located between amino acid 1 and amino acid 24 of the tat protein:

10

Table 2 Tat epitope

AA no	Naturally occurring AAs							
1	M	S						
2	E	D	V					
3	S	Q	P	L	V	A		
4	V	I						
5	D	N						
6	P	H	A					
7	R	N	S	K	E	D		
8	L	I	Q	R	V	M	T	
9	E	D	P					
10	P	S						
11	W							
12	K	N	E	H	L			
13	H	R	Q					
14	P							
15	G	P						
16	S	N	A					
17	Q	K	T					
18	P	H						
19	K	T	S	A	R	P	E	Q
20	T	A	I					
21	A	P	D	V				
22	C	S						
23	T	N	S					
24	N	K	R	Q	A	P	T	

The one letter as well as the three letter codes defining the amino acids in the sequences given throughout this specification are in accordance with International standards and given in textbooks, for instance Lehninger A.L., «Principles of Biochemistry», Worth Publishers Inc., New York, 1982. The amino acids given to the right of the left column, represent the natural variation of the sequence. Our analyses resulted in a sequence containing this modified epitope:

20

C S W V N P R L E P W L H P G S Q P N I T A C T N

5 Wherein NI indicates 2-aminohexanoic acid (Norleucine, abbreviated Nle and NI in the three letter and one letter code, respectively) and the cysteine residues are in an oxidized state, i.e. are forming an intrachain disulphide bridge. Since the Cystein residue to the C-terminal part (in position 22) of the peptide is part of an intramolecular disulfide bond outside this selected epitope, a similar intrapeptide disulphide bond is
 10 formed by placing a Cystein in the N-terminal part of the selected epitope. Another alternative is to form an intermolecular disulphide bond by dimerization of the sequences :

15 W V N P R L E P W L H P G S Q P N I T A C T N
 |
 W V N P R L E P W L H P G S Q P N I T A C T N

A further alternative is dimerization with another epitope selected from Tat. The second epitope on Tat is located between amino acid 35 and 57 in C-terminal direction,
 20 separated from the first epitope by 10 amino acids containing 5 Cys residues in addition to the Cys residue in each of the epitopes. The relatively high number of Cys residues offers a variety of inter- and intramolecular crosslinking possibilities. It is likely that this Cys-rich domain will dominate the immunological exposure of this protein and hence cause a "hiding" of the two relevant epitopes. Selection and modification of the two
 25 adjacent epitopes can expose an essential part of the Tat protein in a more optimal way.

In order to reduce the probability for development of escape mutants, the number of epitopes is further increased and two additional peptide sequences were selected.
 30 These sequences are located on Rev (residues 58-78) and Nef (residues 65-85). The native sequences have been published in Human Retroviruses and AIDS 1999; A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences. Eds. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos :

35

Table 3 Tat epitope

AA no.		Naturally occurring AAs					
35	Q	P	I	L	T	Y	V
36	V	A	C	L			
37	C						
38	F						
39	I	L	Q	M	T		
40	T	N	K	R			
41	K	Q					
42	G	A					
43	L						
44	G	S					
45	I						
46	S	F	Y				
47	Y	N					
48	G						
49	R	K	S				
50	K						
51	K						
52	R						
53	R	K	S	G			
54	Q	R	P				
55	R						
56	R						
57	R	G	S				

Table 4 Rev epitope:

AA no.		Naturally occurring AAs							
58	R	W	Q						
59	I	V	L	F					
60	L	I	P						
61	G	S	N	D	C	V	T	A	R
62	T	A	D	N	S				
63	Y	C	F	R	H	S	V	L	
64	L	V							
65	G	H							
66	R	G							
67	S	P	F	L					
68	A	T	E	Q	V	P	S		
69	E	K	Q	D	N				
70	P	S	A	N					
71	V	N	G	P					
72	P	Q	H	S	L	R	T	D	I
73	L	F	V						
74	Q	L	P	H	D	E			
75	L								
76	P								
77	P	L	E						
78	L	I	V	P					

5

and the

Nef epitope, Table 5 :

AA no.		Naturally occurring AAs				
65	E	G	D	S		
66	N	G	D	E		
67	E	V				
68	A	G				
69	L	F				
70	P					
71	I	V				
72	T	R	K	A	M	
73	P					
74	Q	H				
75	V	L	I			
76	P					
77	L	V	T			
78	R					
79	P					
80	M	V	I			
81	T	D				
82	Y	F	R			
83	K	R				
84	A	G	S	E	Q	
85	A	S	V			

Several modified peptides have been synthesized in order to determine the uniqueness of the sequences as well as their properties for stimulation of the immune system in combination with their specificity and sensitivity as HIV-1 antigens.

DESCRIPTION OF THE INVENTION

The peptides according to the invention are originating from the four different conserved areas of the HIV-1 Tat, Rev and Nef proteins which are described above, having the properties of maintaining the uniqueness (immunogenicity, sensitivity and specificity) of the HIV-1 epitopes. Further the new peptides according to the invention possess no recognized cytotoxic T lymphocyte (CTL) antagonistic effect and shall have at least one potential CTL epitope.

The peptides, according to the invention, which have met the above criteria are selected from the following groups;

Xaa₁ Xaa₂ Xaa₃ Xaa₄Xaa₅ Xaa₆ Xaa₇Leu Glu Pro Trp Xaa₁₂ His Pro Xaa₁₅ Xaa₁₆ Xaa₁₇
Xaa₁₈Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ (SEQ ID NO : 1)

wherein the amino acids of the chain could have the following meanings ;

Xaa in position 1 of the peptide derivative is Met, Ser, Cys or none,

Xaa in position 2 is Glu, Asp, Val, Ser or none

Xaa in position 3 is Ser, Gln, Pro, Leu, Val, Ala, Trp, Tyr or Phe,

5 Xaa in position 4 is Val or Ile,

Xaa in position 5 is Asp, Asn or Ile,

Xaa in position 6 is Pro, His or Ala,

Xaa in position 7 is Arg, Asn, Ser, Lys, Glu or Asp

Xaa in position 12 is Leu, Ile or Nle

10 Xaa in position 15 is Gly or Pro

Xaa in position 16 is Ser, Asn or Ala,

Xaa in position 17 is Gln, Lys or Thr

Xaa in position 18 is Pro or His,

Xaa in Position 19 is Lys, Thr, Ser, Ala, Arg, Pro, Glu, Leu, Ile or Nle

15 Xaa in position 20 is Thr, Ala or Ile

Xaa in position 21 is Ala, Pro, Asp or Val

Xaa in position 22 is Cys or Ser

Xaa in position 23 is Thr, Asn or Ser

Xaa in position 24 is Asn, Lys, Arg, Gln, Ala Pro or Thr

20

the peptide comprises at least six consecutive amino acids of the sequence of SEQ ID

NO : 1,

furthermore two or more of the Cys residues may form part of an intrachain- or
interchain disulphide binding, a -S-(CH₂)_p-S- or a - (CH₂)_p-bridge wherein p = 1-8,

25 optionally intervened by one or more hetero atoms such as O, N or S,

Xaa₁ Xaa₂ Xaa₃ Phe Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀ Xaa₁₁ Xaa₁₂ -Z- Tyr Xaa_i Gly

Xaa₁₅ Lys Lys Arg Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ (SEQ ID NO : 4)

30 wherein the amino acids of the chain have the following meaning;

Xaa in position 1 is Pro, Ile, Leu, Thr, Tyr or Val

Xaa in position 2 is Val, Ala Cys, Leu,

Xaa in position 3 is Cys, Ile, Leu, Val or Nle

Xaa in position 5 is Ile, Leu, Gln, Met or Thr

35 Xaa in position 6 is Thr, Asn, Lys or Arg

- Xaa in position 7 is Lys, Arg or Gln
 Xaa in position 8 is Gly or Ala
 Xaa in position 9 is Leu or Ile
 Xaa in position 10 is Gly, Ser or Ala
 5 Xaa in position 11 is Ile or Gly
 Xaa in position 12 is Ser, Phe or Tyr
 Xaa_i inserted before position 14 is Leu, Ile, Nle
 Xaa in position 15 is Arg, Lys, Ser or Citrulline (Cit)
 Xaa in position 19 is Arg, Lys, Ser, Gly or Cit
 10 Xaa in position 20 is Gln, Arg or Pro
 Xaa in position 21 is Ile or leu
 Xaa in position 22 is Gly, Leu, Ile, Cys or none
 Xaa in position 23 is Gly or none
 wherein the sequence of SEQ ID NO : 4 comprises at least six consecutive amino
 15 acids, -Z- is an optional linker and have the meaning PEG, modified PEG and/or [Gly]_n
 wherein n = 1, 2 or 3,
 furthermore two or more of the Cys residues may form part of an intrachain- or
 interchain disulphide binding, a -S-(CH₂)_p-S- or a - (CH₂)_p-bridge wherein p = 1-8,
 optionally intervened by one or more heteroatoms such as O, N or S,
 20 Xaa₁ Ile Leu Xaa₄ Xaa₅ Xaa₆ Leu Gly Arg Xaa₁₀ Xaa₁₁ -Z- Xaa₁₂ Leu Xaa_i Xaa_j Xaa₁₄
 Xaa₁₅ Xaa₁₆ Xaa₁₇ Xaa₁₈ Xaa₁₉ Leu Pro Pro Leu (SEQ ID NO : 7)
 wherein Xaa in position 1 is Phe, Tyr, Trp or Arg
 25 Xaa in position 4 is Gly, Ser, Asn, Asp, Cys, Val, Thr, Ala, or Arg
 Xaa in position 5 is Thr, Ala, Asp, Asn or Ser
 Xaa in position 6 is Tyr, Cys, Phe, Arg, His, Ser, Val or Leu
 Xaa in position 10 is Ser, Pro, Phe, Leu or Ile
 Xaa in position 11 is Ala, Thr, Glu, Gln, Val Pro or Ser
 30 Xaa in position 12 is Glu, Lys, Gln, Asp, Asn, Tyr, Trp or Phe
 Xaa_i inserted after position 13 is Ser, Pro, Phe, Leu or Ile
 Xaa_i inserted before position 14 is Ala, Thr, Glu, Gln, Val, Pro, or Ser
 Xaa in position 14 is Glu, Lys, Gln, Asp or Asn
 Xaa in position 15 is Pro, Ser, Ala or Asn
 35 Xaa in position 16 is Val, Asn, Gly or Pro

Xaa in position 17 is Pro, Gln, His, Ser, Leu, Arg, Thr, Asp or Ile

Xaa in position 18 is Leu Phe or Val

Xaa in position 19 is Gln, Leu, Pro, His, Asp or Glu

- 5 wherein the sequence of SEQ ID NO : 7 consists of at least six consecutive amino acids, the linker -Z- is optional and have the meaning PEG, modified PEG and/or [Gly]_n wherein n = 1, 2 or 3,

Xaa₁ Leu Val Gly Xaa₅ Pro Xaa₇ Xaa₈ Pro Xaa₁₀ Xaa₁₁ Pro -Z-[Arg]_m Xaa_i Xaa₁₃ Xaa₁₄ -
 10 Pro Xaa₁₆ Xaa₁₇ Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (SEQ ID NO : 10)

wherein the Xaa in position 1 is Lys or Arg

Xaa in position 5 is Phe or Leu

Xaa in position 7 is Ile or Val

- 15 Xaa in position 8 is Thr, Arg, Lys, Ala or Met

Xaa in position 10 is Gln or His

Xaa in position 11 is Val, Leu or Ile

Xaa_i inserted before position 13 is Leu

Xaa in position 13 is Leu, Val or Thr

- 20 Xaa in position 14 is Arg or Citrulline (Cit)

Xaa in position 16 is Met, Val, Ile or Nle, Leu

Xaa in position 17 is Thr or Asp

Xaa in position 18 is Tyr, Phe or Arg

Xaa in position 19 is Lys or Arg

- 25 Xaa in position 20 is Ala, Gly, Ser, Glu or Gln

Xaa in position 21 is Ala, Ser or Val

wherein the sequence of SEQ ID NO : 10 consists of at least six consecutive amino acids, the linker -Z- is optional and have the meaning PEG, modified PEG and/or [Gly]_n

- 30 wherein n = 1, 2 or 3 and independently from n, m in [Arg]_m is = 0, 1, 2 or 3,
 the terminal ends of the sequences may be free carboxyl- or amino groups, amides, acyls, acetyls or salts thereof, and/or the said peptide sequences are immobilized to a solid support.

The new peptide sequences have the potential to serve as a good antigen wherein the antigen comprises at least one peptide selected from the group of sequences of SEQ ID NO : 1, SEQ ID NO : 4, SEQ ID NO : 7 or SEQ ID NO : 10 . The antigenicity may be adapted through adjusting the ratio or concentration of different peptides or size of the peptides by for instance dimerization or polymerization and/or immobilization to a solid phase. The antigen comprises one or more polypeptide sequences, according to the invention, which could be either linked by a bridge for instance a disulphide bridge between the Cys residues of the chains or bridges like C₁-C₈ alkylen possibly intervened by one or more heteroatoms like O, S, or N or preferably they are unlinked. The chains may be immobilized to a solid phase in monomeric, dimeric or oligomeric forms. Further amino acids may be added to the ends in order to achieve an «arm» to facilitate immobilization.

PEG is polyethylene glycol (HO(CH₂CH₂O)_a H and can be part of the linker -Z-, optionally PEG is modified by a dicarboxylic acid (HO(CH₂CH₂O)_a CO(CH₂)_b COOH) or a terminal carboxylic group (HO(CH₂CH₂O)_{a-1}CH₂COOH) where a = 1-10 and b = 2-6, prior to linking.

The linker -Z- can either consist of PEG, modified PEG, or a combination thereof and/or one or more Gly residues combined. Alternatively the linker -Z- can consist of a Gly-bridge [Gly]_n where n = 1, 2 or 3.

All amino acids in the peptides of the invention can be in both D- or L-form, although the naturally occurring L- form is preferred.

The C- and N-terminal ends of the peptide sequences could deviate from the natural sequences by modification of the terminal NH₂-group and/or COOH-group, they may for instance be acylated, acetylated, amidated or modified to provide a binding site for a carrier or another molecule.

The peptides according to the invention are consisting of 6 to 50 amino acids, preferably between 10 and 30 amino acids. They are covering all natural variation of amino acids in the identified positions.

The polypeptide antigen according to the invention is either in a free or in a carrier-bound form. The carrier or solid phase to which the peptide is optionally bound can be selected from a wide variety of known carriers. It should be selected with regard to the intended use of the immobilized polypeptide as a diagnostic antigen or as an immunizing component in a vaccine.

Examples of carriers that can be used for e.g. diagnostic purposes are magnetic beads or latex of co-polymers such as styrene-divinyl benzene, hydroxylated styrene-divinyl benzene, polystyrene, carboxylated polystyrene, beads of carbon black, non-activated or polystyrene or polyvinyl chloride activated glass, epoxy-activated porous magnetic glass, gelatine or polysaccharide particles or other protein particles, red blood cells, mono- or polyclonal antibodies or fab fragments of such antibodies.

According to a further embodiment of the present invention, the antigens may form part of a vaccine possibly combined with carriers, adjuvants or combined with other immunostimulating elements such as canarypox virus carrying the *env* gene. Examples of carriers and/or adjuvants for vaccine purposes are other proteins such as human or bovine serum albumin and keyhole limpet haemocyanin and fatty acids. Immunostimulatory materials may be divided into three groups; adjuvants, carriers for antigens and vehicles. Examples of adjuvants include aluminum hydroxyd, aluminum salts, saponin, muramyl di and tripeptides, monophosphoryl lipid A, palmitic acid, *B.pertussis* and various cytokines including the Th1 cytokine IL-12 and IL-1. A number of protein toxins can be used to carry passenger proteins across cellular membranes into the cytosol, which are useful in developing CTL vaccines. Carriers include bacterial toxoids such as inactivated tetanus and cholera toxins, genetically detoxified bacterial toxins such as heat labile enterotoxin from *E.coli*, fatty acids, live vectors such as polio chimeras and hybrid proteins that form particulates for example yeast retrotransposon hybrid TY particles and HBcAg particles. Vehicles which are frequently occurring components in modern vaccines are consisting of mineral oil emulsion, Freund's complete and incomplete adjuvant, vegetable oil emulsions, nonionic block co-polymer surfactants, squalene or squalane, lipopeptides, liposomes and biodegradable microspheres. Two novel adjuvants which possess significant potential for the development of new vaccines include an oil-in-water microemulsion (MF59) and polymeric microparticles. Any substance that can enhance the immunogenicity of the

antigen may be used and several further alternatives of carriers or adjuvants are given in the US or European Pharmacopoeia.

A suitable formulation of the antigen for immunostimulatory uses may also comprise
5 interferons such as INF- γ , antiviral chemokines or haematopoietic growth factors such as granulocyte macrophage growth factor.

Another approach in order to enhance the stimulation and absorption in for instance the intestine is to administer the peptides of the invention, with small peptides such as di, tri-
10 or tetrapeptides. These peptides can be administered in addition to or in combination with the peptides of the invention. Preferably the peptides are administered together with the tripeptide YGG, consisting of amino acids in the D- or L-forms, preferably in the D-form.

Recent approaches to non-parenteral delivery of vaccines, for instance via mucosa
15 include; gene fusion technology to create non-toxic derivatives of mucosal adjuvants, genetically inactivated antigens with a deletion in an essential gene, coexpression of an antigen and a specific cytokine that is important in the modulation and control of a mucosal immune response, and genetic material itself that would allow DNA or RNA uptake and its endogenous expression in the host's cells.

20 One approach for developing durable responses where cell-mediated immunity is required, is to vaccinate with plasmid DNA encoding one or more specific antigen(s).

In order to protect against HIV infection, vaccines should induce both mucosal and
25 systemic immune responses and could be administered by any convenient route, parenterally or non-parenterally, such as subcutaneously, intracutaneously, intravenously, intramuscularly, perorally, mucosally or intranasally for example.

In a preferred embodiment of the vaccine according to the present invention it
30 comprises antigens containing peptides selected from at least one of the groups of the SEQ ID NO: 1, 4, 7 and 10, more preferred the different peptides occur in equal amounts.

In a further preferred embodiment the vaccine composition contains the antigens ;
35

FVIHRLEPWLHPGSQH NITASTN - NH₂ (SEQ ID NO :14)

and

5

RLVGFPVKPQVPGLLRPLTYKAA - NH₂ (SEQ ID NO :15).

10 The sequences can activate the cellular immune system, and contribute with CTL-epitopes. The amino acid changes implemented within the frame of the CTL-epitopes are designed to achieve enhanced binding. Other amino acid changes have been conducted in order to facilitate the synthesis of the peptide and/or increase the solubility of the peptide.

15 A method for detecting antibodies, induced by HIV-1 or HIV-1 specific peptides or proteins, in a sample of body fluid using the present antigens is a further embodiment of the invention. Also immunoassay kit designed for this detection and antibodies capable of selectively reacting with the said antigens are encompassed by the present invention.

20 DESCRIPTION OF THE PREPARATION OF THE PEPTIDES

The peptides of the invention can be produced by any known method of producing a linear amino acid sequence, such as recombinant DNA techniques. A nucleic acid sequence that encodes one or more peptides of the invention or a multimer of the said peptides, is introduced into an expression vector. Suitable expression vectors are for instance plasmids, cosmids, viruses and YAC (yeast artificial chromosome) which
25 comprise necessary control regions for replication and expression. The expression vector may be stimulated to expression in a host cell. Suitable host cells are for example bacteria, yeast cells and mammal cells. Such techniques are well known in the art and described for instance by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989. Other well-
30 known techniques are degradation or synthesis by coupling of one amino acid residue to the next one in liquid phase or preferably on a solid phase (resin) for instance by the so-called Merrifield synthesis. See for instance Barany and Merrifield in the Peptides, Analysis, Synthesis, Biology, Vol.2, E. Gross and Meinhofer, Ed. (Acad.Press, N.Y.,

1980), Kneib-Coronier and Mullen Int. J. Peptide Protein Res., 30, p.705-739 (1987) and Fields and Noble Int.J.Peptide Protein Res., 35, p.161-214 (1990).

5 In case a linked or cyclic peptide is desired, the amino acid sequence is subjected to a chemical oxidation step in order to cyclize or link the two cysteine residues within one or between two peptide sequences, when the appropriate linear amino acid sequences are synthesized, see Akaji et al., Tetrahedron Letter, 33, 8, p.1073-1076, 1992.

GENERAL DESCRIPTION OF SYNTHESIS

10 All peptide derivatives prepared in the Examples given below were synthesized on a Milligen 9050 Peptide Synthesizer using a standard program. The resin used was Tenta Gel P RAM with a theoretical loading of 0,20 meq/g (RAPP POLYMER GmbH, Tübingen). The final product of the synthesis was dried *in vacuo* overnight. The peptide was then cleaved from the resin by treatment with 90% trifluoroacetic acid in
15 the presence of ethandithiol (5%) and water (5%) as scavengers (1,5 hours at RT). Then the resin was filtered and washed on filter with additional trifluoro acetic acid (100%) (2 x 20 ml). The combined filtrates were evaporated *in vacuo* (water bath at RT) and the residue was triturated with ethyl ether (200 ml) and the precipitated product filtered off. The solid was promptly dissolved on filter with glacial acetic acid (100 ml)
20 and added to 1,5 l of 20% acetic acid in methanol and treated with 0,1 M solution of iodine in methanol until a faint brown colour remained. Then Dowex 1 x 8 ion exchange in acetate form (15g) (Bio-Rad, Richmond, CA) was added and the mixture filtered. The filtrate was evaporated and the residue freeze-dried from acetic acid. The product was then purified by reversed phase liquid chromatography on a column filled with
25 Kromasil® 100 - 5 C8 (EKA Nobel, Surte, Sweden) in a suitable system containing acetonitrile in 0,1 % trifluoro acetic acid water solution. The samples collected from the column were analyzed by analytical high performance liquid chromatography (HPLC) (Beckman System Gold, USA) equipped with a Kromasil® 100 - 5 C8 Column (EKA Nobel, Surte, Sweden). Fractions containing pure substance were pooled, the solvent
30 was evaporated and the product freeze-dried from acetic acid. The final HPLC analysis was performed on final product, and the structure of the peptide was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

All amino acids used during the synthesis were L-amino acids and they were protected with a fluorenyl methoxy-carbonyl group at the α -amino function. The side chains were protected as follows:

- 5 Cys (Trt), Gln(Trt), Glu(OtBu), Thr(tBu).

The abbreviations, within the brackets are :

Trt = triphenyl methyl

t-Bu = tert. Butyl

- 10 OtBu = tert. Butyl ester

The amino acid derivatives were supplied by Bachem AG, Switzerland.

EXAMPLE 1

Preparation of C S W V N P R L E P W N I H P G S Q H N I T A C T N - NH₂

- 15 (SEQ ID NO : 2). The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The peptide was then cyclized by oxidation with I₂. The peptide was dissolved in acetic acid/methanol (1:4) and 0,1 M I₂ in methanol was added. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-
20 MS).

Purity (HPLC) : more than 97% (single impurity less than 1%)

Molecular weight (free base) : 2746.2

EXAMPLE 2

- 25 Preparation of F V I P R L E P W N I H P G S Q P N I T A C T N - NH₂ (SEQ ID NO : 3).

The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

- 30 Purity (HPLC) : more than 97% (single impurity less than 1%)

Molecular weight (free base) : 2538,0

EXAMPLE 3

Preparation of Y L L F L T K G L G I S G G G Y N I G C i t K K R C i t Q I L G - NH₂
(SEQ ID NO : 5). The peptide is synthesized in amide form, from the corresponding
starting materials according to the general description of synthesis. The purity is
5 determined by HPLC analysis and the structure is confirmed by amino acid analysis
and mass spectrometry (LDI-MS).

EXAMPLE 4

Preparation of Y L N I F L T R G L G I S G G G Y N I G C i t K K R C i t Q I C G - NH₂
10 (SEQ ID NO : 6). The peptide was synthesized in amide form, from the corresponding
starting materials according to the general description of synthesis. The purity was
determined by HPLC analysis and the structure was confirmed by amino acid analysis
and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 97% (single impurity less than 1%)

15 Molecular weight (free base) : 3097,7

EXAMPLE 5

Preparation of R I L S T Y L G R I S G G G W L S A E P V P L Q L P P L - NH₂
(SEQ ID NO : 8). The peptide was synthesized in amide form, from the corresponding
20 starting materials according to the general description of synthesis. The purity was
determined by HPLC analysis and the structure was confirmed by amino acid analysis
and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 97% (single impurity less than 1%)

Molecular weight (free base) : 2990,6

EXAMPLE 6

Preparation of R I L S T Y L G R I S G G G Y L S A E P V P L Q L P P L - NH₂
(SEQ ID NO : 9). The peptide is synthesized in amide form, from the corresponding
starting materials according to the general description of synthesis. The purity is
30 determined by HPLC analysis and the structure is confirmed by amino acid analysis
and mass spectrometry (LDI-MS).

EXAMPLE 7

Preparation of KLVGFVPVKPQVPGGGRLLCITPNITYKAA-NH₂
 (SEQ ID NO : 11). The peptide is synthesized in amide form, from the corresponding
 starting materials according to the general description of synthesis. The purity is
 5 determined by HPLC analysis and the structure is confirmed by amino acid analysis
 and mass spectrometry (LDI-MS).

EXAMPLE 8

Preparation of RLVGFVPVKPQVPGGGRLRLPTYKAA-NH₂
 10 (SEQ ID NO : 12). The peptide was synthesized in amide form, from the corresponding
 starting materials according to the general description of synthesis. The purity was
 determined by HPLC analysis and the structure was confirmed by amino acid analysis
 and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 97% (single impurity less than 1%)

15 Molecular weight (free base) : 2790,4

Molecular formula : C₁₃₀H₂₁₇O₃₉N₂₉

EXAMPLE 9**Dimerisation via disulphide bridge.**

20 The peptide sequences of the Example 2 are linked via an oxidation step to form a
 dipeptide wherein the cysteine residues form a disulphide bridge. The bridge is formed
 in either ways;

A) Oxidation with I₂. The peptides (equal amounts if different) are dissolved in acetic
 acid/methanol (1:4) and 0,1 M I₂ in methanol is added yielding a mixture of the

25 homodimer,

or

B) Oxidation via [Cys(Spy)²²]-SEQ ID NO : 3. 2,3mM of the peptide of SEQ ID NO : 3
 dissolved in 2 M AcOH (aq) and 2-propanol (1:1) is treated with 2,2 dithiodipyridin (3
 eqv) to yield [Cys(Spy)²²]-SEQ ID NO : 3. [Cys(Spy)²²]-SEQ ID NO : 3 is dissolved in 10
 30 mM NH₄Oac (aq pH=6, 5) and methanol (5:2) to yield the dimer of SEQ ID NO : 13.

The purity of the resulting dimer is determined by HPLC analysis and the structure is
 confirmed by amino acid analysis and mass spectrometry (LDI-MS).

EXAMPLE 10

Preparation of F V I H R L E P W L H P G S Q H N I T A S T N - NH₂ (SEQ ID NO :14).

The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 98% (single impurity less than 1%)

Molecular weight (free base) : 2540,2

EXAMPLE 11

Preparation of R L V G F P V K P Q V P G L L R P L T Y K A A - NH₂ (SEQ ID NO :15).

The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 99% (single impurity less than 1%)

Molecular weight (free base) : 2520,3

EXAMPLE 12 – REFERENCE EXAMPLE

Preparation of a nativ tat1 sequence ; M E S V D P R L E P W K H P G S Q P K T A C T N -NH₂ (SEQ ID NO :16). The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 97% (single impurity less than 1%)

Molecular weight (free base) : 2708,1

EXAMPLE 13 – REFERENCE EXAMPLE

Preparation of a nativ tat2 sequence ; Q V C F I T K G L G I S Y G R K K R R Q R R R -NH₂ (SEQ ID NO :17). The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 94%

Molecular weight (free base) : 2806,4

EXAMPLE 14 – REFERENCE EXAMPLE

Preparation of a nativ Nef sequence ; E E V G F P V R P Q V P L R P M T Y K A A - NH₂ (SEQ ID NO :18). The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The
5 purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 95%

Molecular weight (free base) : 2384,8

10 EXAMPLE 15

A vaccine comprising the peptides of the SEQ ID NO : 3 and 12 is prepared. The freeze-dried peptides are dissolved in sterile water at a final concentration of 4 mg/ml. A preparation of a granulocyte-macrophage-colony stimulating factor (GM-CSF) is also prepared, according to the manufacturers directions for use, to a final concentration of
15 0,3 mg/ml. The two solutions are administered intracutaneously. A typical injection dose is 100 µl.

EXAMPLE 16

A vaccine comprising the peptides of the SEQ ID NOS : 14 and 15 is prepared. The
20 freeze-dried peptides are dissolved in sterile water at a final concentration of 4 mg/ml. A preparation of a granulocyte-macrophage-colony stimulating factor (GM-CSF) is also prepared, according to the manufacturers directions for use, to a final concentration of 0,3 mg/ml. The two solutions are administered intracutaneously. A typical injection dose is 100 µl.

25

EXAMPLE 17

An antigen solution or suspension is mixed with equal parts of Freund's adjuvant of Behring, complete or incomplete, and is then finely emulsified by being drawn up into, and vigorously pressed out of, an injection syringe, or with a homogenator. The
30 emulsion should remain stable for at least 30 minutes. The antigen-adjuvant emulsions is best injected subcutaneously as a depot.

EXAMPLE 18**Immunoassay for detection of antibodies induced by HIV-1.**

The magnetic particle reagents are to be prepared according to the manufacturers recommended protocol. Dynal AS, is the manufacturer of the Dynabeads, which are employed. The magnetic particles coated with ligand are called Reagent 1. A peptide according to the invention is covalently coupled to the pre-activated surface of the magnetic particles. It is also possible to physically absorb the peptide to the surface of the magnetic particles. The concentration of particles in Reagent 1 is within the range from 1 mg/ml to 15 mg/ml. The particle size varies between 0,2 μ m to 15 μ m. The concentration of peptides is within the range from 0,01 mg/mg particle to 1 mg/mg particle.

The anti human Ig Alkaline Phosphatase (AP) conjugated antibody reagent is prepared according to the recommended protocol of Dako AS. This protocol is a standard procedure in this field. This reagent is called Reagent 2.

The substrate solution phenolphthaleine-monophosphate is to be prepared according to the recommended protocol of Fluka AG. This protocol is a standard procedure in this field. The substrate solution is called Reagent 3.

The washing and incubation buffer which is used is standard 0,05M tris-base buffer with the following additional compounds; Tween 20 (0,01% to 0,1%), glycerol (0,1% to 10%) and sodium chloride (0,2% to 0,1%).

The assay procedure comprises an incubation step wherein 1 drop of Reagent 1 is mixed with 2 drops of washing buffer in each well. After mixing, 30 μ l of sample is added and the solution is incubated for 5 minutes. The magnetic particles can be trapped by a magnet and the liquid removed, before the magnet is separated. Then the wells are washed twice in 4 drops of washing solution, before incubation with Reagent 2. 1 drop of Reagent 2 is added with 2 drops of washing buffer and the solution is incubated for 5 minutes. The magnetic particles can be trapped by a magnet and the liquid removed, before the magnet is separated. Then the washing step is repeated before incubation with Reagent 3. 2 drops of Reagent 3 is added to each well and the solution is incubated for 3 minutes. The results can be read against a white background. Positive results are red (3+ = strong red) whereas negative results are clearly light yellow/brown solutions as obtained in the negative control.

The immunoassay kit could be used in detection of antibodies, induced either by HIV virus or HIV-specific peptides or proteins, for instance the peptides of the present invention.

5 **EXAMPLE 19**

Therapeutic or prophylactic vaccine.

At least one of the polypeptides of the invention, selected from the group of sequences SEQ ID NO : 1, SEQ ID NO : 4, SEQ ID NO : 7 or SEQ ID NO : 10 can form antigens and constitute the active principle of a prophylactic or therapeutic vaccine intended to
10 provide protection against the human immunodeficiency virus type 1 (HIV-1). The vaccine may include compounds having beneficial effects in protecting or stimulating the hosts immune system (human being or vertebrate animal) for instance interleukins, interferons, granulocyte macrophage growth factors, haematopoietic growth factors or similar. Preferably the vaccine composition further contain an adjuvant or vehicle, more
15 preferably the adjuvant or vehicle is Monophosphoryl Lipid A (MPL ®) possibly with alum, Freund's adjuvant (complete or incomplete) or aluminum hydroxyd. The optimal amount of adjuvant/vehicle will depend on the type(s) which is chosen.

The peptides of the invention might be modified by C-terminal addition of a single fatty
20 acid such as a single palmitoyl chain to form a lipopeptide vaccine. Further the lipopeptides can be introduced into liposome membranes by the freeze-thaw method resulting in liposomes bearing the peptide ligands on their surface.

The peptide or vaccine formulation can be freeze-dried prior to storage. The freeze-
25 dried peptides can be dissolved in sterile water to a final concentration of 0,1-100 mg/ml. The vaccine may be stored preferably at low temperature, in ampoules containing one or more dosage units, ready for use. A typical dosage unit of the peptide according to the invention is within the concentration range : 0,05 µg-1mg per kg bodyweight, preferably within 0, 15 µg-0.15 mg per kg body weight. Persons skilled in
30 the art will appreciate that a suitable dose will depend on the body weight of the patient, the type of disease, severity of condition, administration route and several other factors. When used as a therapeutic vaccine, the vaccine might be administered up to 12 times, through injections. Further boosters might follow and can in some cases take place throughout the patients life. In preparation of an injection solution the

peptides are dissolved in sterile water at a final concentration of 1 mg/ml per peptide. Typically an injection volume is 100 μ l to 200 μ l (2 x 100 μ l). The peptide is preferably co-administered with a suitable adjuvant and/or a granulocyte-macrophage growth factor for instance Leucomax® «Shering Plough» made within a concentration range of
5 from 0,1 mg/ml to 1 mg/ml, or according to the manufacturers recommendations for use. Particularly preferred is a combination therapy where the present peptides are administered together with the peptides as described in the published International patent application no. PCT/NO00/00075 and/or the co-pending Norwegian patent application no. 2000 4413 . These peptides may be administered simultaneously or
10 sequentially. Suitable administration may be intracutane, subcutane, intravenous, peroral, intramuscular, intranasal, mucosal or any other suitable route. Booster administrations may be required in order to maintain protection. For persons skilled in the art it will be understood that the vaccine compositions according to the invention are useful not only in prevention of infection, but also in treatment of infection.

15

No toxic effects of the peptides according to the invention, are observed when injected in mice in a dosage of 100 μ g per kg body weight.

20

The above Examples are only meant as illustrating the invention. It must be understood that a person skilled in the art can modify the peptides, antigens and vaccines herein described without deviating from the concept and scope of this invention as set forth in the claims.

25

PATENT CLAIMS

1. Peptide characterized in that it comprises at least one amino acid sequence selected from the groups of amino acid sequences :

5

Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Xaa₆ Xaa₇ Leu Glu Pro Trp Xaa₁₂ His Pro Xaa₁₅ Xaa₁₆ Xaa₁₇
Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ (SEQ ID NO : 1)

wherein the amino acids of the chain could have the following meanings ;

10 Xaa in position 1 of the peptide derivative is Met, Ser, Cys or none,

Xaa in position 2 is Glu, Asp, Val, Ser or none

Xaa in position 3 is Ser, Gln, Pro, Leu, Val, Ala, Trp, Tyr or Phe,

Xaa in position 4 is Val or Ile,

Xaa in position 5 is Asp, Asn or Ile,

15 Xaa in position 6 is Pro, His or Ala,

Xaa in position 7 is Arg, Asn, Ser, Lys, Glu or Asp

Xaa in position 12 is Leu, Ile or Nle

Xaa in position 15 is Gly or Pro

Xaa in position 16 is Ser, Asn or Ala,

20 Xaa in position 17 is Gln, Lys or Thr

Xaa in position 18 is Pro or His,

Xaa in Position 19 is Lys, Thr, Ser, Ala, Arg, Pro, Glu, Leu, Ile or Nle

Xaa in position 20 is Thr, Ala or Ile

Xaa in position 21 is Ala, Pro, Asp or Val

25 Xaa in position 22 is Cys or Ser

Xaa in position 23 is Thr, Asn or Ser

Xaa in position 24 is Asn, Lys, Arg, Gln, Ala Pro or Thr

the peptide comprises at least six consecutive amino acids of the sequence of SEQ ID NO : 1,

30

Xaa₁ Xaa₂ Xaa₃ Phe Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀ Xaa₁₁ Xaa₁₂ -Z- Tyr Xaa_i Gly
Xaa₁₅ Lys Lys Arg Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ (SEQ ID NO : 4)

wherein the amino acids of the chain have the following meaning;

35 Xaa in position 1 is Pro, Ile, Leu, Thr, Tyr or Val

- Xaa in position 2 is Val, Ala Cys, Leu,
 Xaa in position 3 is Cys, Ile, Leu, Val or Nle
 Xaa in position 5 is Ile, Leu, Gln, Met or Thr
 Xaa in position 6 is Thr, Asn, Lys or Arg
 5 Xaa in position 7 is Lys, Arg or Gln
 Xaa in position 8 is Gly or Ala
 Xaa in position 9 is Leu or Ile
 Xaa in position 10 is Gly, Ser or Ala
 Xaa in position 11 is Ile or Gly
 10 Xaa in position 12 is Ser, Phe or Tyr
 Xaa_i inserted before position 14 is Leu, Ile, Nle
 Xaa in position 15 is Arg, Lys, Ser or Citrulline (Cit)
 Xaa in position 19 is Arg, Lys, Ser, Gly or Cit
 Xaa in position 20 is Gln, Arg or Pro
 15 Xaa in position 21 is Ile or leu
 Xaa in position 22 is Gly, Leu, Ile, Cys or none
 Xaa in position 23 is Gly or none
 wherein the sequence of SEQ ID NO : 4 comprises at least six consecutive amino
 acids, -Z- is an optional linker and have the meaning PEG, modified PEG and/or [Gly]_n
 20 wherein n = 1, 2 or 3,

Xaa₁ Ile Leu Xaa₄ Xaa₅ Xaa₆ Leu Gly Arg Xaa₁₀ Xaa₁₁ -Z- Xaa₁₂ Leu₁₃ Xaa_i Xaa_i Xaa₁₄
 Xaa₁₅ Xaa₁₆ Xaa₁₇ Xaa₁₈ Xaa₁₉ Leu Pro Pro Leu (SEQ ID NO : 7)

- 25 wherein Xaa in position 1 is Phe, Tyr, Trp or Arg
 Xaa in position 4 is Gly, Ser, Asn, Asp, Cys, Val, Thr, Ala, or Arg
 Xaa in position 5 is Thr, Ala, Asp, Asn or Ser
 Xaa in position 6 is Tyr, Cys, Phe, Arg, His, Ser, Val or Leu
 Xaa in position 10 is Ser, Pro, Phe, Leu or Ile
 30 Xaa in position 11 is Ala, Thr, Glu, Gln, Val, Pro or Ser
 Xaa in position 12 is Glu, Lys, Gln, Asp, Asn, Tyr, Trp or Phe
 Xaa_i inserted after position 13 is Ser, Pro, Phe, Leu or Ile
 Xaa_i inserted before position 14 is Ala, Thr, Glu, Gln, Val, Pro, or Ser
 Xaa in position 14 is Glu, Lys, Gln, Asp or Asn
 35 Xaa in position 15 is Pro, Ser, Ala or Asn

Xaa in position 16 is Val, Asn, Gly or Pro

Xaa in position 17 is Pro, Gln, His, Ser, Leu, Arg, Thr, Asp or Ile

Xaa in position 18 is Leu Phe or Val

Xaa in position 19 is Gln, Leu, Pro, His, Asp or Glu

- 5 wherein the sequence of SEQ ID NO : 7 consists of at least six consecutive amino acids, the linker -Z- is optional and have the meaning PEG, modified PEG and/or [Gly]_n, wherein n = 1,2 or 3,

- 10 Xaa₁ Leu Val Gly Xaa₅ Pro Xaa₇ Xaa₈ Pro Xaa₁₀ Xaa₁₁ Pro -Z- [Arg]_m Xaa_i Xaa₁₃ Xaa₁₄ Pro Xaa₁₆ Xaa₁₇ Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (SEQ ID NO : 10)

wherein the Xaa in position 1 is Lys or Arg

Xaa in position 5 is Phe or Leu

Xaa in position 7 is Ile or Val

- 15 Xaa in position 8 is Thr, Arg, Lys, Ala or Met

Xaa in position 10 is Gln or His

Xaa in position 11 is Val, Leu or Ile

Xaa_i inserted before position 13 is Leu

Xaa in position 14 is Leu, Val or Thr

- 20 Xaa in position 15 is Arg or Citrulline (Cit)

Xaa in position 17 is Met, Val, Ile or Nle, Leu

Xaa in position 18 is Thr or Asp

Xaa in position 19 is Tyr, Phe or Arg

Xaa in position 20 is Lys or Arg

- 25 Xaa in position 21 is Ala, Gly, Ser, Glu or Gln

Xaa in position 22 is Ala, Ser or Val

wherein the sequence of SEQ ID NO : 10 consists of at least six consecutive amino acids, the linker -Z- is optional and have the meaning PEG, modified PEG and/or [Gly]_n, wherein n = 1, 2 or 3 and independently from n, m in [Arg]_m is 0, 1, 2 or 3 ,

30

the terminal ends of the sequences may be free carboxyl- or amino groups, amides, acyls, acetyls or salts thereof,

two or more of the Cys residues may form part of an intrachain- or interchain disulphide binding, a -S-(CH₂)_p-S- or a -(CH₂)_p-bridge wherein p = 1-8 optionally intervened by one

or more heteroatoms such as O, N and S and/or the said peptide sequences are immobilized to a solid support.

2. Peptide according to claim 1, characterized in that
5 the amino acid sequence of SEQ ID NO : 1 is selected from the groups of SEQ ID NO :
2, SEQ ID NO : 3 and SEQ ID NO : 14.

3. Peptide according to claim 1, characterized in that
the amino acid sequence of SEQ ID NO : 4 is selected from the groups of SEQ ID NO :
10 5 and SEQ ID NO : 6.

4. Peptide according to claim 1, characterized in that
the amino acid sequence of SEQ ID NO : 7 is selected from the groups of SEQ ID NO :
8 and SEQ ID NO : 9.

15 5. Peptide according to claim 1, characterized in that
the amino acid sequence of SEQ ID NO : 10 is selected from the groups of SEQ ID NO
: 11, SEQ ID NO : 12 and SEQ ID NO : 15.

20 6. Antigen, characterized in that it comprises at least one peptide
according to claim 1.

7. Antigen according to claim 6, characterized in that it comprises at
least one peptide selected from at least one of the groups SEQ ID NO : 1, SEQ ID NO :
25 4, SEQ ID NO : 7 and SEQ ID NO : 10.

8. Vaccine composition, characterized in that
it comprises an antigen according to claim 6 with a pharmaceutically acceptable diluent
and optionally an adjuvant, carrier and/or vehicle and optionally additional
30 immunostimulatory compound(s).

9. Vaccine composition according to claim 8, characterized in that it
comprises at least one peptide selected from at least one of the groups of SEQ ID NO
: 1, SEQ ID NO : 4, SEQ ID NO : 7 and SEQ ID NO : 10.

10. Vaccine composition according to claim 9, characterized in that it comprises the peptides of the SEQ ID NO : 14 and SEQ ID NO : 15.
11. Vaccine composition according to the claims 8-10, characterized in
5 that the peptides are dissolved in a sterile water solution and the optional immunostimulatory compound is a granulocyte macrophage colony stimulating factor.
12. Vaccine composition according to the claims 8-11, characterized in
that the composition comprises an adjuvant selected from the group Monophosphoryl-
10 Lipid A (MPL®), Freund's complete or incomplete adjuvant or aluminum hydroxyd.
13. Vaccine composition, characterized in that an antigen according to claim 6 is formulated as a lipopeptide and/or a liposome formulation.
- 15 14. A method of detecting antibodies, induced by a HIV or HIV-specific peptides or proteins, in a sample of body fluid, characterized in that subjecting the said sample to an immunoassay, wherein the antigen(s) is/are selected from the peptides of the claims 1, 2, 3, 4 and 5.
- 20 15. An immunoassay kit for the detection of antibodies, induced by a HIV or HIV-specific peptides or proteins, in a sample of body fluid, characterized in that the diagnostic antigen is a peptide of any one of the previous claims 1 to 5.
16. Antibody, characterized in that it is capable of selectively reacting
25 with the antigen of the claims 6 and 7.

SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Bionor Immuno AS
(B) STREET: Strømdalsjordet 4, P.O.Box 1893 Gulset
10 (C) CITY: 3703 Skien

(E) COUNTRY: Norway

(F) POSTAL CODE (ZIP): N-3705

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(ii) TITLE OF INVENTION: HIV regulatory and auxiliary peptides, antigens, vaccine compositions, immunoassay and a method of detecting antibodies induced by HIV.

20 (iii) NUMBER OF SEQUENCES: 18

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM Compatible
25 (C) OPERATING SYSTEM: Windows 2000
(D) SOFTWARE: Word 7.0

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER:

30

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22-24 amino acids
35 (B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

40

(iii) HYPOTHETICAL: No

(v) FRAGMENT TYPE : internal

45 (ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

(D) OTHER INFORMATION: /note= " Xaa in position 1 is Met, Ser, Cys or none

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 2

(D) OTHER INFORMATION: /note= " Xaa in position 2 is Glu, Asp, Val, Ser or none

5

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 3

(D) OTHER INFORMATION: /note= " Xaa in position 3 is Ser, Gln, Pro, Leu, Val, Ala,

10 Trp, Tyr or Phe

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 4

(D) OTHER INFORMATION: /note= " Xaa in position 4 is Val or Ile

15

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

(D) OTHER INFORMATION: /note= " Xaa in position 5 is Asp, Asn or Ile

20

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 6

(D) OTHER INFORMATION: /note= " Xaa in position 6 is Pro, His or Ala

25

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 7

(D) OTHER INFORMATION: /note= " Xaa in position 7 is Arg, Asn, Ser, Lys, Glu or Asp

30

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 12

(D) OTHER INFORMATION: /note= " Xaa in position 12 is Leu, Ile or Nle

35

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 15

(D) OTHER INFORMATION: /note= " Xaa in position 15 is Gly or Pro

40

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 16

(D) OTHER INFORMATION: /note= " Xaa in position 16 is Ser, Asn or Ala

45

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 17

(D) OTHER INFORMATION: /note= " Xaa in position 17 is Gln, Lys or Thr

50

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 18

(D) OTHER INFORMATION: /note= " Xaa in position 18 is Pro or His

55

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 19

(D) OTHER INFORMATION: /note= " Xaa in position 19 is Lys, Thr, Ser, Ala, Arg, Pro,

5 Glu, Leu, Ile or Nle

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 20

10 (D) OTHER INFORMATION: /note= " Xaa in position 20 is Thr, Ala, or Ile

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 21

15 (D) OTHER INFORMATION: /note= " Xaa in position 21 is Ala, Pro, Asp or Val

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 22

20 (D) OTHER INFORMATION: /note= " Xaa in position 22 is Cys or Ser

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 23

25 (D) OTHER INFORMATION: /note= " Xaa in position 23 is Thr, Asn or Ser

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 24

30 (D) OTHER INFORMATION: /note= " Xaa in position 24 is Asn, Lys, Arg, Gln, Ala, Pro or Thr

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1

35 (D) OTHER INFORMATION: /note= " Cys in position 1 may form part of a disulphide bridge

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 22

40 (D) OTHER INFORMATION: /note= " Cys in position 22 may form part of a disulphide bridge

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1 :

Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Xaa₆ Xaa₇ Leu Glu Pro Trp Xaa₁₂ His Pro Xaa₁₅ Xaa₁₆ Xaa₁₇

50 1 5 10 15

Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄

20

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 1...22
(D) OTHER INFORMATION: /note= " Cys in position 1 and 22 may form part of an intrachain disulphide bridge

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2 :

Cys Ser Trp Val Asn Pro Arg Leu Glu Pro Trp Nle His Pro Gly Ser Gln His Nle Thr
1 5 10 15 20

Ala Cys Thr Asn

(2) INFORMATION FOR SEQ ID NO: 3 :

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3 :

Phe Val Ile Pro Arg Leu Glu Pro Trp Nle His Pro Gly Ser Gln Pro Nle Thr Ala Cys
1 5 10 15 20

Thr Asn

(2) INFORMATION FOR SEQ ID NO: 4 :

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24-27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

- 5 ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= " Xaa in position 1 is Pro, Ile, Leu, Thr, Tyr or Val
- 10 ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= " Xaa in position 2 is Val, Ala Cys, Leu,
- 15 ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= " Xaa in position 3 is Cys, Ile, Leu, Val or Nle
- 20 ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION: /note= " Xaa in position 5 is Ile, Leu, Gln, Met or Thr
- 25 ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= " Xaa in position 6 is Thr, Asn, Lys or Arg
- 30 ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note= " Xaa in position 7 is Lys, Arg or Gln
- 35 ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 8
(D) OTHER INFORMATION: /note= " Xaa in position 8 is Gly or Ala
- 40 ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= " Xaa in position 9 is Leu or Ile
- 45 ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 10
(D) OTHER INFORMATION: /note= " Xaa in position 10 is Gly, Ser or Ala
- 50 ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 11
(D) OTHER INFORMATION: /note= " Xaa in position 11 is Ile or Gly
- 55 ix) FEATURE:

- (A) NAME/KEY: Modified-site
 (B) LOCATION: 12
 (D) OTHER INFORMATION: /note= " Xaa in position 12 is Ser, Phe or Tyr

5 ix) FEATURE:

- (A) NAME/KEY: Modified-site
 (B) LOCATION: Xaa₁ inserted before position 14
 (D) OTHER INFORMATION: /note= " Xaa₁ inserted before the position 14 is Leu, Ile, Nle

10 ix) FEATURE:

- (A) NAME/KEY: Modified-site
 (B) LOCATION: 15
 (D) OTHER INFORMATION: /note= " Xaa in position 15 is Arg, Lys, Ser or Citrulline

15 ix) FEATURE:

- (A) NAME/KEY: Modified-site
 (B) LOCATION: 19
 (D) OTHER INFORMATION: /note= " Xaa in position 19 is Arg, Lys, Ser, Gly or Citrulline

20 ix) FEATURE:

- (A) NAME/KEY: Modified-site
 (B) LOCATION: 20
 (D) OTHER INFORMATION: /note= " Xaa in position 20 is Gln, Arg or Pro

ix) FEATURE:

- 25 (A) NAME/KEY: Modified-site
 (B) LOCATION: 21
 (D) OTHER INFORMATION: /note= " Xaa in position 21 is Ile or leu

ix) FEATURE:

- 30 (A) NAME/KEY: Modified-site
 (B) LOCATION: 22
 (D) OTHER INFORMATION: /note= " Xaa in position 22 is Gly, Leu, Ile, Cys or none

ix) FEATURE:

- 35 (A) NAME/KEY: Modified-site
 (B) LOCATION: 23
 (D) OTHER INFORMATION: /note= " Xaa in position 23 is Gly or none

ix) FEATURE:

- (A) NAME/KEY: Modified-site
 (B) LOCATION: 12..13
 (D) OTHER INFORMATION: /note= " optionally inserted linker -Z-

40

ix) FEATURE:

- (A) NAME/KEY: Modified-site
 (B) LOCATION: 2, 3 and 22
 (D) OTHER INFORMATION: /note= " Cys in the positions 2, 3 and 22 may form part of a
 45 disulphide bridge

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4 :

Xaa₁ Xaa₂ Xaa₃ Phe Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀ Xaa₁₁ Xaa₁₂ -Z- Tyr Xaa₁ Gly
 50 1 5 10 15

Xaa₁₅ Lys Lys Arg Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃
 20

(2) INFORMATION FOR SEQ ID NO: 5 :

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 12..13
(D) OTHER INFORMATION: /note= " inserted Gly-bridge of 3 residues

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5 :

Tyr Leu Leu Phe Leu Thr Lys Gly Leu Gly Ile Ser Gly Gly Gly Tyr Nle Gly Cit Lys Lys
1 5 10 15 20
Arg Cit Gln Ile Leu Gly
25

(2) INFORMATION FOR SEQ ID NO: 6 :

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 12..13
(D) OTHER INFORMATION: /note= " inserted Gly-bridge of 3 residues

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 27
(D) OTHER INFORMATION: /note= " Cys in position 27 may form part of a disulphide
bridge

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Tyr Leu Nle Phe Leu Thr Arg Gly Leu Gly Ile Ser Gly Gly Gly Tyr Nle Gly Cit Lys Lys
1 5 10 15 20
Arg Cit Gln Ile Cys Gly
25

(2) INFORMATION FOR SEQ ID NO: 7 :

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25-28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: /note= " Xaa in position 1 is Phe, Tyr, Trp or Arg

ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= " Xaa in position 4 is Gly, Ser, Asn, Asp, Cys, Val, Thr, Ala, or Arg

ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /note= " Xaa in position 5 is Thr, Ala, Asp, Asn or Ser

ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= " Xaa in position 6 is Tyr, Cys, Phe, Arg, His, Ser, Val or Leu

ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 10
- (D) OTHER INFORMATION: /note= " Xaa in position 10 is Ser, Pro, Phe, Leu or Ile

ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 11
- (D) OTHER INFORMATION: /note= " Xaa in position 11 is Ala, Thr, Glu, Gln, Val, Pro or Ser

ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 12
- (D) OTHER INFORMATION: /note= " Xaa in position 12 is Glu, Lys, Gln, Asp, Asn, Tyr, Trp or Phe

ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: Xaa, inserted after position 13
- (D) OTHER INFORMATION: /note= " Xaa, inserted after position 13 is Ser, Pro, Phe, Leu or Ile

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: Xaa_i inserted before position 14

5 (D) OTHER INFORMATION: /note= " Xaa_i inserted before position 14 is Ala, Thr, Glu,
Gln, Val, Pro, or Ser

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 14

10 (D) OTHER INFORMATION: /note= " Xaa in position 14 is Glu, Lys, Gln, Asp or Asn

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 15

15 (D) OTHER INFORMATION: /note= " Xaa in position 15 is Pro, Ser, Ala or Asn

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 16

20 (D) OTHER INFORMATION: /note= " Xaa in position 16 is Val, Asn, Gly or Pro

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 17

25 (D) OTHER INFORMATION: /note= " Xaa in position 17 is Pro, Gln, His, Ser, Leu, Arg,
Thr, Asp or Ile

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 18

30 (D) OTHER INFORMATION: /note= " Xaa in position 18 is Leu Phe or Val

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 19

35 (D) OTHER INFORMATION: /note= " Xaa in position 19 is Gln, Leu, Pro, His, Asp or Glu

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 11..12

40 (D) OTHER INFORMATION: /note= " optionally inserted linker -Z-

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 4

45 (D) OTHER INFORMATION: /note= " Cys in position 4 and 6 may form part of a
disulphide bridge

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7 :

Xaa₁ Ile Leu Xaa₄ Xaa₅ Xaa₈ Leu Gly Arg Xaa₁₀ Xaa₁₁ -Z- Xaa₁₂ Leu₁₃ Xaa₁ Xaa₁ Xaa₁₄

1

5

10

15

Xaa₁₅ Xaa₁₆ Xaa₁₇ Xaa₁₈ Xaa₁₉ Leu Pro Pro Leu
20 25

(2) INFORMATION FOR SEQ ID NO: 8 :

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
10 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No
15
ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 11..12
(D) OTHER INFORMATION: /note= " inserted [Gly]_n bridge, wherein n = 3
20
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8 :

Arg Ile Leu Ser Thr Tyr Leu Gly Arg Ile Ser Gly Gly Gly Trp Leu Ser Ala Glu Pro Val
1 5 10 15 20
25 Pro Leu Gln Leu Pro Pro Leu
25

INFORMATION FOR SEQ ID NO: 9 :

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No
40
ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 11..12
(D) OTHER INFORMATION: /note= " inserted [Gly]_n bridge, wherein n = 3
45
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9 :

Arg Ile Leu Ser Thr Tyr Leu Gly Arg Ile Ser Gly Gly Gly Tyr Leu Ser Ala Glu Pro Val
50 1 5 10 15 20
Pro Leu Gln Leu Pro Pro Leu
25

(2) INFORMATION FOR SEQ ID NO: 10 :

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22-29 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= " Xaa in position 1 is Lys or Arg

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION: /note= " Xaa in position 5 is Phe or Leu

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note= " Xaa in position 7 is Ile or Val

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 8
(D) OTHER INFORMATION: /note= " Xaa in position 8 is Thr, Arg, Lys, Ala or Met

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 10
(D) OTHER INFORMATION: /note= " Xaa in position 10 is Gln or His

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 11
(D) OTHER INFORMATION: /note= " Xaa in position 11 is Val, Leu or Ile

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: Xaa_i inserted before position 13
(D) OTHER INFORMATION: /note= " Leu is inserted before position 13

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 13
(D) OTHER INFORMATION: /note= " Xaa in position 13 is Leu, Val or Thr

ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 14

(D) OTHER INFORMATION: /note= " Xaa in position 14 is Arg or Cit

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 16

(D) OTHER INFORMATION: /note= " Xaa in position 16 is Met, Val, Ile, Leu or Nle

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 17

(D) OTHER INFORMATION: /note= " Xaa in position 17 is Thr or Asp

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 18

(D) OTHER INFORMATION: /note= " Xaa in position 18 is Tyr, Phe or Arg

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 19

(D) OTHER INFORMATION: /note= " Xaa in position 19 is Lys or Arg

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 20

(D) OTHER INFORMATION: /note= " Xaa in position 20 is Ala, Gly, Ser, Glu or Gln

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 21

(D) OTHER INFORMATION: /note= " Xaa in position 21 is Ala, Ser or Val

ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 12...(Leu_iXaa₁₃)

(D) OTHER INFORMATION: /note= " optionally a linker -Z- and/or an [Arg]_m bridge is inserted between Xaa₁₂ and (Leu_iXaa₁₃), wherein m is 0,1, 2 or 3.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10 :

Xaa₁ Leu Val Gly Xaa₅ Pro Xaa₇ Xaa₈ Pro Xaa₁₀ Xaa₁₁ Pro -Z- [Arg]_m Leu_i Xaa₁₃

1 5 10

Xaa₁₄ Pro Xaa₁₆ Xaa₁₇ Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁

15 20

(2) INFORMATION FOR SEQ ID NO: 11 :

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: both

5 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:

- 10 (A) NAME/KEY: Modified-site
- (C) LOCATION: 12...(Leu_iXaa₁₃....)
- (D) OTHER INFORMATION: /note= " the linker -Z- is [Gly]_n wherein n is 3 and an [Arg]_m wherein m is 1.

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11 :

Lys Leu Val Gly Phe Pro Val Lys Pro Gln Val Pro Gly Gly Gly Arg Leu Leu Cit Pro Nle
 1 5 10 15 20
 20 Thr Tyr Lys Ala Ala
 25

(2) INFORMATION FOR SEQ ID NO: 12 :

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: both

30 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

35 ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (E) LOCATION: 12...(Leu_iXaa₁₃)
- (F) OTHER INFORMATION: /note= " the linker -Z- is [Gly]_n wherein n is 3 and an [Arg]_m wherein m is 1.

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12 :

Arg Leu Val Gly Phe Pro Val Lys Pro Gln Val Pro Gly Gly Gly Arg Leu Leu Arg Pro Leu
 45 1 5 10 15 20
 Thr Tyr Lys Ala Ala
 25

50 (2) INFORMATION FOR SEQ ID NO: 13 :

(i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 44 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: dimeric peptide

(iii) HYPOTHETICAL: No

5 ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: disulphide-bond between position 20 in SEQ ID NO : 3 and position 20
in SEQ ID NO : 3

10 (2) INFORMATION FOR SEQ ID NO: 14 :

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 22 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: both

20 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14 :

25 Phe Val Ile His Arg Leu Glu Pro Trp Leu His Pro Gly Ser Gln His Nle Thr Ala Ser Thr Asn
1 5 10 15 20

(2) INFORMATION FOR SEQ ID NO: 15 :

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: double

35 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15 :

Arg Leu Val Gly Phe Pro Val Lys Pro Gln Val Pro Gly Leu Leu Arg Pro Leu Thr Tyr Lys Ala Ala
1 5 10 15 20

45 (2) INFORMATION FOR SEQ ID NO: 16 :

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 24 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

55

(iii) HYPOTHETICAL: No, tat1 nativ sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16 :

5 Met Glu Ser Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro Lys Thr Ala Cys Thr
1 5 10 15 20
Asn

10 (2) INFORMATION FOR SEQ ID NO: 17 :

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 amino acids

(B) TYPE: amino acid

15 (C) STRANDEDNESS: double

(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

20 (iii) HYPOTHETICAL: No, tat2 nativ sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17 :

25 Gln Val Cys Phe Ile Thr Lys Gly Leu Gly Ile Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg
1 5 10 15 20

(2) INFORMATION FOR SEQ ID NO: 18 :

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: double

35 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No, nativ Nef sequence

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18 :

Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Ala Ala
1 5 10 21

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 March 2002 (14.03.2002)

PCT

(10) International Publication Number
WO 02/20555 A3

(51) International Patent Classification⁷: C07K 14/16.
A61K 39/21, G01N 33/569

(21) International Application Number: PCT/NO01/00363

(22) International Filing Date:
3 September 2001 (03.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2000 4412 4 September 2000 (04.09.2000) NO

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,

CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI,
SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU,
ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
TG).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

(88) Date of publication of the international search report:
6 June 2002

*For two-letter codes and other abbreviations, refer to the "Guidance
Notes on Codes and Abbreviations" appearing at the beginning
of each regular issue of the PCT Gazette.*



WO 02/20555 A3

(54) Title: HIV PEPTIDES FROM TAT, REV AND WEF CONSERVED REGIONS AND THEIR APPLICATION AS E.G. VACCINE COMPONENTS

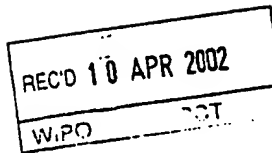
(57) Abstract: The present invention comprises novel and modified peptides capable of inducing a HIV-1 specific immune response without antagonizing the cytotoxic T-cell activity in order to achieve an effective prophylactic and therapeutic vaccine against HIV. The peptides are based on conserved regions of HIV Tat and Rev, regulatory proteins and Nef, auxiliary proteins. Antigens in free- or carrier-bound form comprising at least one of the said peptides, vaccine compositions containing at least one of the antigens, immunoassay kits and a method of detecting antibodies induced by HIV or HIV specific peptides using such antigens, are described.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)



Applicant's or agent's file reference 104830/HNY	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/NO 01/00363	International filing date (day, month, year) 3 Sept 2001	(Earliest) Priority Date (day, month, year) 4 Sept 2000
Applicant BIONOR IMMUNO AS ET AL		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☒ Unity of invention is lacking (See Box II).

4. With regard to the title,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

HIV PEPTIDES FROM TAT, REV AND WEF CONSERVED REGIONS AND THEIR APPLICATION AS E.G. VACCINE COMPONENTS.

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 35.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NO 01/00363 ..

A. CLASSIFICATION OF SUBJECT MATTER		
IPC7: C07K 14/16, A61K 39/21, G01N 33/569 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC7: C07K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
SE,DK,FI,NO classes as above		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
EPO-INTERNAL, BIOSIS, EBI		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9902185 A1 (THYMON L.L.C.), 21 January 1999 (21.01.99), page 4, line 24 - page 7; page 11, line 15 - page 22 --	1-3,6,8,9, 11-16
X	Nature Medicine, Volume 1, No. 9, September 1966, G. Goldstein: "HIV-1 Tat protein as a potential AIDS vaccine", page 960 - page 964, figure 2, page 963 --	1-3,6,8,9, 11-16
X	WO 9927958 A2 (ISTITUTO SUPERIORE DI SANITA), 10 June 1999 (10.06.99), claims 10, 11, pages 1-5, 35-36 --	1-3,6,8,9, 11-16
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
2 April 2002		04-04-2002
Name and mailing address of the ISA Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86		Authorized officer Carl-Olof Gustafsson/BS Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NO 01/00363

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9415634 A1 (RATH, MATTHIAS), 21 July 1994 (21.07.94), claims --	1
A	FR 2773156 A1 (BIOVACS INC), 2 July 1999 (02.07.99), example 1 --	1-3, 6, 8, 9, 11-16
A	WO 9627389 A1 (NEOVACS), 12 Sept 1996 (12.09.96), claim 20 --	1-3, 6, 8, 9, 11-16
P, X	WO 0078969 A1 (THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES), 28 December 2000 (28.12.00), page 8, line 20 - line 32; page 46, line 28 -- -----	1-3, 6, 8, 9, 11-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO01/00363

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see next sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO01/00363

According to PCT Rule 13.2, an international application shall relate to one invention only or a group of inventions linked by one or more of the same or corresponding "special technical features", i.e. features that define a contribution which each of the inventions makes over the prior art.

The claimed invention relates to HIV peptides and their application in vaccines, assays etc.. A first group of peptides are obtained from Tat. However such peptides are known in the art and have been used for the improvement of vaccines and assays, see e.g. WO9902185, pages . Therefore the application fails, a posteriori, to comply with PCT-rule 13.2.

The following inventions have been found:

Invention 1. Claims: 1, 2, 3, 6, 8, 9 and 11-16 partially.
Peptides, vaccines, antibodies etc. selected from Tat residues 1-24 with conserved amino acids ...LEPWXHP... and corresponding DNA. SEQ IDs 1-3, 14 and 16.

Invention 2. Claims: 1, 2, 3, 6, 8, 9 and 11-16 partially.
Peptides, vaccines, antibodies etc. selected from Tat residues 35-57 with the sequence ...FXXXXXXXX-YXGXKKR... and corresponding oligonucleotides. SEQ IDs 5,6, 7 and 17.

Invention 3. Claims: 1, 4, 6, 8, 11-16 (partially)
Peptides, vaccines, antibodies etc. selected from Rev residues 58-78 with the sequence XILXXXLGRXX-XLXXXXXXXXLPPLL and corresponding DNA SEQ IDs 8 and 9

Invention 4. Claims: 1, 5, 10-16 (partially).
Peptides, vaccines, antibodies etc. selected from Nef residues 67-85 with the sequence ...PXXPXXRP... and corresponding DNA. SEQ IDs 11, 12, 15 and 18.

Invention 5. Claims: 1, 7, 9
Combinations of peptides according to examples 15 and 16.

All peptides are defined by only a few "conserved" amino acids and the rest of the peptide is variable. Support for all variable amino acids claimed are not found in the examples although most (all?) are known in HIV. Consequently the search has been limited to the general aspects of Tat 1-24 and 35-57 peptides for vaccine or assay purpose and the selected peptides having SEQ IDs according to the description.

.../...

INTERNATIONAL SEARCH REPORT

International application No.
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The search has been limited to inventions 1 and 2.

If additional searches are requested, the applicant should bear in mind that the respective peptides could be searched but that combinations of peptides cannot be adequately searched - except for the specific combinations of defined peptides revealed in the examples.

INTERNATIONAL SEARCH REPORT
Information on patent family members

28/01/02

International application No.
PCT/NO 01/00363 ..

Patent document cited in search report			Publication date	Patent family member(s)		Publication date
WO	9902185	A1	21/01/99	AU	8392798 A	08/02/99
				EP	0994724 A	26/04/00
				JP	2001509368 T	24/07/01
				US	5891994 A	06/04/99
				US	6193981 B	27/02/01
WO	9927958	A2	10/06/99	AP	200001828 D	00/00/00
				AU	2412699 A	16/06/99
				BR	9814725 A	03/10/00
				CN	1283121 T	07/02/01
				EP	1035865 A	20/09/00
				IT	1297090 B	03/08/99
				IT	RM970743 A	01/06/99
				JP	2001524531 T	04/12/01
				PL	341818 A	07/05/01
				TR	200001553 T	00/00/00
WO	9415634	A1	21/07/94	NONE		
FR	2773156	A1	02/07/99	AU	1568499 A	19/07/99
				AU	1939799 A	19/07/99
				BR	9814473 A	23/10/01
				EP	1041888 A	11/10/00
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				JP	2001527029 T	25/12/01
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				WO	9933872 A	08/07/99
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				BR	9607659 A	15/12/98
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				DK	814834 T	13/11/00
				EP	0814834 A,B	07/01/98
				SE	0814834 T3	
				JP	11501310 T	02/02/99
				PL	322143 A	05/01/98
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				US	6200575 B	13/03/01
				CA	2215483 A	12/09/96
				CN	1181020 A	06/05/98
				ES	2150107 T	16/11/00
				FR	2731355 A,B	13/09/96
				PT	814834 T	31/01/01
WO	0078969	A1	28/12/00	AU	4082300 A	09/01/01

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